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Volumetric measurement of microscopic open craters in the evaluation of dental

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Chapter 1 introduces the concept of root resorption in orthodontics and the aims of this series of study. It defines the subject and describes the complexity of this phenomenon. Although the literature has noted that root resorption does have a high incidence in the naturally occurring dentition as well as treated cases, the amount of destruction is usually self-limiting and does not undermine the form and longevity of a normal functional occlusion.

The structure of cementum is complex and its properties have been demonstrated to affect the extent of tissue destruction during physiologic and orthodontic tooth movement. Although it has been compared to bone, the structure of cementum has subtle differences. It serves an important role as part of the periodontium during tooth movement in orthodontics. The remodeling of bone by giant multi-nucleate cells has brought about inadvertent damage to the root surface and this damage could extend beyond cementum to reach the dentine structures if the resorption is severe. A layer of precementum normally forms a barrier to this destruction but could be pathologically breached by mechanical and/or chemical means. Resorption defects of various sizes have been previously documented by different techniques. These techniques are further described.

The features of root resorption and factors that pertain to this topic are highlighted. The single cell unit that causes this tissue destruction is identified. The notion of chemical and molecular control of root resorption is introduced. Besides the activities involving tissue destruction, the repair potential of root resorption is also illustrated. This repair process is related to the cellular activities at tissue level as well as the mechanical factors involving orthodontic forces. It is hypothesized that all resorptions will be repaired once the cause of root resorption has ceased. However other authors claim that all resorptions would heal only provided that the resorbed surface area does not exceed the unresorbed one.

Methodologies of root resorption studies using the SEM are reviewed. The strength and weaknesses of such studies are highlighted. As a consequence of the flaws in their methodologies, many of these studies do not come into agreement with each other. The aims of this study are reiterated. We aim to reproduce a digital 3-D replica of root resorption crater, develop a protocol to allow volumetric analysis of root resorption craters on controlled samples of human premolar teeth, calibrate the commercial software *Analysis Pro 3.1* on accuracy of volumetric analysis, evaluate the effect of orthodontic force magnitude on volume of root resorption craters, and identify the sites that may be predisposed to root resorption by volumetric analysis of root resorption craters.

Chapter 2 describes the new methodology involving volumetric measurement of open resorption craters on the surface of human root cementum. Previous attempts at quantification of such craters are described. The difficulty of obtaining a true 3-D measurement of such defects has prevented the understanding of this topic in its entirety. With specially developed software *Analysis Pro 3.1*, this difficulty is overcome. Using the SEM as a capturing device, stereo images of each resorption crater taken at 6 degrees apart ($\pm 3^\circ$) are imported into the software. They are superimposed and color-coded to produce an anaglyph to allow for quick 3-D referencing. A 3-D digital model of the crater is also produced. Further image processing involved their conversion into 8-bit grayscale images, shading correction to eliminate the innate surface curvature of the cementum, holes filling and binary thresholding to allow generation of a depth map profile. Image analysis involves manipulation of the data collected from the depth map to obtain volumetric quantification of each individual crater. It is demonstrated that resorption craters could be measured down to sizes of $1.6 \pm 0.15 \times 10^5 \mu\text{m}^3$. In a preliminary sample of 20 premolars, heavy force (225 g) cause more resorption than light force (25 g) when a buccal tipping force of 28 days is applied.

Chapter 3 documents the calibration process of this new software *Analysis Pro 3.1* in its use to obtain volumetric measurement of open resorption craters. Most reports on volumetric measurements in medical sciences involve quantification of enclosed or geometrically symmetric regions. The difficulty in obtaining an accurate volume of an irregularly shaped open crater is recognised. Using the Vickers hardness tester, pyramidal indentations of known volumetric dimension are made on 4 selected metallic rods. These rods are chosen based on their relative hardness and are matched to the size of the human premolars to mimic the innate curvature of the cementum surfaces. These known volumetric dimensions are then correlated to the estimated volumes obtained by the software. It is demonstrated that the software is successful in estimating the volumes of pyramidal indentations in metallic rods. Non-uniform plastic deformation that occurs in softer materials during indentation distorts the calculated results. The estimates obtained by the software even for distorted indentations caused by non-uniform plastic deformation have high degrees of reproducibility and accuracy. It is noted that most previous studies involving root resorption are comparative in nature. This calibration process allows absolute volumetric quantification in the microanalysis of small biologic samples.

Chapter 4. After verification of the quantification process, a sample of 36 human premolars is collected after an experimental period involving the application of light and heavy orthodontic forces over 28 days. Using the methodology

described in chapters 2 and 3, the images of the craters are collected, processed and analysed. The mean volume of the resorption crater in light-force group is 3.49-fold greater than the control group, and the heavy-force group 11.59-fold more than control group ($p < 0.001$). The heavy force group has 3.31-fold greater total resorption volume than light force group ($p < 0.001$). Buccal cervical and lingual apical regions demonstrate significantly more resorption craters than the other regions ($p < 0.001$). There is more resorption by volume in the heavy force group as compared to the light group and controls. Although there is more resorption recorded in the light group, the difference in amount of resorption between the light and control groups are not of significant statistical difference. There is significantly more resorption on the buccal cervical and lingual apical regions of the root surfaces than other regions suggesting that high-pressure zones may be more susceptible to resorption after 28 days of force application.

Chapter 5 explores the extent of root resorption in the areas undergoing tissue compression and tension in the sample collected in chapter 4. Buccal and lingual surfaces are divided in to 3 equal regions: cervical, middle and apical. The root surface area of the 3 regions is documented with straight-on images captured with the SEM. Quantification of resorption craters using volumetric analysis is performed from stereo images taken at $\pm 3^\circ$ as previously described in chapters 2, 3 and 4. This is correlated to the amount of surface area under compression/tension. The buccal cervical region has 8.16-fold more root resorption in the heavy group as compared to the light force group ($p < 0.01$). All other regions do not seem to have any significant difference within the force levels. In the experimental teeth, there is more root resorption in the high compression regions as compared to the other regions ($p < 0.01$). Although there is more resorption per unit area on the lingual apical region as compared to the buccal cervical regions, it is not of any statistical significance. Regions under compression have more root resorption than regions under tension. There is more resorption in regions under heavy compression when compared to regions under light compression ($p < 0.01$). There is also more root resorption in regions under heavy tension when compared to regions under light tension ($p < 0.01$). This study documents in detail the extent of root resorption in areas under compression and tension on the root surface which has not been done before. The valuable information obtained allows a greater understanding of this phenomenon and also the biology of tooth movement in respect to tissue damage under light and heavy compression and tensile forces.

root resorption between light and heavy forces. However most studies do not quantify the true extent of what is light or heavy force. They somehow alluded that the varying results obtained in these studies are due to the inter-individual differences. It is recognised that root resorption has a multi-factorial aetiology and this explanation provided seems to be logical to a certain extent. After the elimination of confounding factors in patient selection, it is demonstrated that it is possible to obtain a relatively unbiased result clearly indicating the merits of using light over heavy orthodontic forces. Moreover this present series of studies have highlighted the importance of obtaining an accurate 3-D quantification of root resorption in order to understand this topic to a fuller extent.

The root surface areas undergoing compression demonstrates higher level of resorption due to the occlusion of the blood vessels. Higher compressive forces show greater extent of tissue damage which may indicate that there are a higher percentage of occluded blood vessels. This predisposes to the formation of hyalinized tissue at the compression interface which leads to the recruitment of giant multi-nucleated cells which have been identified as the cells directly involved in hard tissue destruction. Higher tensile forces seem to also demonstrate greater extent of root resorption than lighter tensile forces. The greater tissue respond created at this high tensile interface seem to have resulted in greater extent of tissue damage.

The future direction following this series of studies is discussed. With the correlation of the results obtained in this series of studies to physical properties such as hardness and elasticity of cementum, as well as to the mineral composition of cementum, would provide a better understanding of this complex idiopathic phenomenon in orthodontics. Further extension into the molecular and genetic control of such tissue destruction would help suggest better means of management and perhaps also the prevention of root resorption.